Short Note

Microsatellite DNA markers provide informative genetic data for studies on New Zealand *Cyanoramphus* parakeets

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Abstract

Several *Cyanoramphus* parakeet species have been the focus of research projects and intense conservation efforts in recent years. However, their evolution and population dynamics are still not fully understood. We have carried out new tests to investigate the utility of Forbes' parakeet (*C. forbesi*) microsatellite DNA markers in two other *Cyanoramphus* species (*C. auriceps* and *C. novaezelandiae novaezelandiae*). Our results show the presence of species or population specific alleles in these birds, and clear differentiation between *Cyanoramphus* populations. This suggests that our microsatellite DNA markers could provide informative genetic data on the evolution and population dynamics of *Cyanoramphus* parakeets in general.

Keywords: *Cyanoramphus* - genetic diversity - genetic differentiation -microsatellite. - phylogenetics - speciation.

Introduction

At least 8 living species and subspecies of *Cyanoramphus* parakeets are found in New Zealand territories (see Boon et al. 2001 for a detailed list). The taxonomy of the *Cyanoramphus* group has not been entirely resolved, and there have been some disputes (Triggs & Daugherty 1996; Taylor 1998; Kearvell *et al.* 2003). Present understanding of the relationships between *Cyanoramphus* taxa comes from early studies of their morphology (such as Taylor 1975; Nixon 1981, 1982), complemented by later genetic work using allozymes (Triggs & Daugherty 1996) and mitochondrial DNA analyses (Boon *et al.* 2000, 2001).

Morphological identification of *Cyanoramphus* parakeets relies mainly on head plumage patterns, and interspecific hybridisation in the past and present has made this complicated (see Taylor 1975, 1976; Nixon 1982; Taylor *et al*. 1986;

Chan *et al*. 2006). The current phylogeny of *Cyanormphus* parakeets is based on mitochondrial DNA control region sequences (Boon *et al.* 2000, 2001). These show a number of minor discrepancies with the earlier genetic phylogeny based on allozyme electrophoresis data (Triggs & Daugherty 1996). These differences include the placement of Forbes' parakeets (*C. forbesi*) and orange-fronted parakeets (*C. malherbi*), and phylogeographic subdivision of yellow-crowned parakeets (*C. auriceps*; Boon *et al*. 2000, 2001). To account for these apparent differences, Boon *et al*. (2001) proposed various possible explanations such as cytonuclear disequilibrium or inadvertent amplification of nuclear copies of mitochondrial DNA sequences. In order to test these competing hypotheses adequately, new nuclear markers with greater resolving power than allozymes are needed. DNA microsatellites represent highly prospective candidates for this purpose. Suitable microsatellite DNA markers have already been isolated from the Forbes' parakeet genome and used in a previous interspecific hybridisation study (Chan *et al.* 2006). Tests have shown that these loci are also amplifiable by Polymerase Chain Reaction (PCR) in other *Cyanoramphus* species (Chan *et al*. 2005).

The population genetic status of most *Cyanoramphus* parakeets is unknown, because only the population genetics of the Mangere Island parakeet population (a mixed population of Forbes' parakeets *C. forbesi*, Chatham Island red-crowned parakeets *C. novaezelandiae chathamensis*, and hybrids) and the Rangatira population of Chatham Island red-crowned parakeets (*C. n. chathamensis*) have been studied to date (Chan *et al*. 2006). In this study, microsatellite markers are used to differentiate between species and populations of two further species of *Cyanoramphus* parakeets (*C. auriceps* and *C. novaezelandiae novaezelandiae*). Our aim is to provide evidence for the wider potential of these markers for population genetic and phylogenetic studies in further *Cyanoramphus* species. Red-crowned parakeets (*C. n. novaezelandiae*) and yellow-crowned parakeets (*C. auriceps*) both have relatively large populations in New Zealand. A comprehensive study of these populations will allow us to compare the levels of genetic variation in various species of *Cyanoramphus* parakeets, particularly those on Mangere Island.

Methods

DNA was extracted from 18 Eglinton Valley yellow-crowned parakeets (*C. auriceps*) and 15 Poor Knights Islands red-crowned parakeets (*C. n. novaezelandiae*) from blood samples deposited in the New Zealand Frozen Tissue Collection at Victoria University of Wellington, using High Pure PCR Template Preparation Kit (Roche). Microsatellites were genotyped as described in Chan et al. (2005), and the status of novel alleles were confirmed by DNA sequencing. Microsatellite genotypic data at 5 loci (*Cfor0809*, *Cfor1415*, *Cfor2021*, *Cfor2829*, and *Cfor3031*) for 250 Mangere Island parakeets (Forbes', Chatham Island red-crowned and hybrid parakeets) and 35 Rangatira Chatham Island red-crowned parakeets (Chan et al. 2006) were already available as a comparative standard.

The number of alleles, observed and expected heterozygosities at each locus in the populations were calculated using the Microsatellite Analyser (MSA) software (version M3.15; Dieringer & Schlötterer 2002). Deviation form Hardy-Weinberg equilibrium (HWE) was tested by the Markov chain method (Guo & Thompson 1992) as implemented in the GENE-

POP software (version 3.4; Raymond & Rousset 1995).

To assess the level of differentiation between the populations, the estimator of F_{ST} (Weir & Cockerham 1984) was calculated using MSA. Analysis of molecular variance (AMOVA; Excoffier et al. 1992) as implemented in the GeneticStudio software (version 2.01; Dyer & Sork 2001) was also used. Genetic distances between populations, based on the proportion of shared alleles (Bowcock et al. 1994), was estimated with the MSA software, and the resulting distance matrix was analysed by principal coordinates analysis implemented in the software PCO (Anderson 2003).

Results

The Rangatira population shows the lowest allelic diversity in terms of number of alleles among the four populations surveyed (Table 1). But the Mangere Island and Rangatira populations both showed low levels of heterozygosity compared with Eglinton Valley and Poor Knights Islands parakeet populations. Even though smaller numbers of individuals were sampled from Eglinton Valley and Poor Knights Islands, these two populations appear more genetically diverse in number of alleles and expected heterozygosity. Two loci *Cfor1415* and *Cfor3031* are found to deviate from

Table 1. Microsatellite loci characteristics in four parakeet populations. N, n, H_O, H_E, and P(HWE) indicate the number of alleles found, sample size, observed heterozygosity, expected heterozygosity, and P values for Hardy-Weinberg equilibrium tests respectively.

Population	Locus	N	Average N	Allele sizes range (bp)	H_O	H_E	Average H_O	Average H_E	P(HWE)
Mangere	*Cfor0809*	4	4.6	183-203	0.08	0.09	0.38	0.43	0.42
(n = 250)	*Cfor1415*	7		211-227	0.76	0.73			0.00
	Cfor2021	2		233-239	0.26	0.26			1.00
	Cfor2829	3		219-231	0.38	0.40			0.06
	Cfor3031	7		233-247	0.41	0.65			0.00
Rangatira	*Cfor0809*	3	3.2	187-203	0.37	0.36	0.42	0.42	1.00
(n = 35)	*Cfor1415*	5		211-227	0.74	0.72			0.57
	Cfor2021	1		233	0.00	0.00			-
	Cfor2829	2		219-221	0.46	0.39			0.40
	Cfor3031	5		233-241	0.54	0.65			0.15
Eglinton	*Cfor0809*	4	5.6	187-207	0.44	0.62	0.67	0.67	0.10
(n = 18)	*Cfor1415*	6		205-233	1.00	0.81			0.06
	Cfor2021	4		229-237	0.56	0.52			0.53
	Cfor2829	7		213-249	0.72	0.75			0.10
	Cfor3031	7		233-247	0.61	0.67			0.36
Poor Knights	*Cfor0809*	3	5.6	187-195	0.67	0.57	0.68	0.67	0.71
(n = 15)	*Cfor1415*	11		199-255	1.00	0.87			0.07
	Cfor2021	3		229-237	0.33	0.40			0.33
	Cfor2829	4		213-221	0.67	0.64			0.12
	Cfor3031	7		229-241	0.73	0.85			0.05

Table 2. Allele frequencies at loci *Cfor0809, Cfor1415, Cfor2021, Cfor2829, and Cfor3031* in the Managere Island (Forbes', Chatham Island red-crowned and hybrid parakeets), Rangatira (Chatham Island red-crowned parakeets), Eglinon Valley (yellow-crowned parakeets), and Poor Knight Islands (red-crowned parakeets) populations.

Locus	Allele (bp)	Allele frequencies in populations			
		Mangere	Rangatira	Eglinton	Poor Knights
Cfor0809	183	0.002	-	-	-
	187	0.006	0.057	0.528	0.133
	191	0.954	0.786	0.333	0.600
	195	-	-	0.083	0.267
	203	0.038	0.157	-	-
	207	-	-	0.056	-
Cfor1415	199	-	-	-	0.067
	201	-	-	-	0.067
	203	-	-	-	0.067
	205	-	-	0.083	0.033
	207	-	-	-	0.033
	209	-	-	0.167	0.033
	211	0.122	0.429	0.250	0.233
	213	0.118	0.257	0.222	0.267
	215	0.022	0.057	0.250	0.133
	217	0.428	-	-	-
	221	0.050	0.171	-	0.033
	223	-	-	0.028	-
	225	0.026	-	-	0.033
	227	0.234	0.086	-	-
Cfor2021	229	-	-	0.028	0.100
	233	0.844	1.000	0.667	0.767
	235	-	-	0.139	-
	237	-	-	0.167	0.133
	239	0.156	-	-	-
Cfor2829	213	-	-	0.028	0.033
	215	-	-	0.028	0.167
	219	0.752	0.743	0.333	0.533
	221	0.120	0.257	0.361	0.267
	223	-	-	0.139	-
	231	0.128	-	-	-
	245	-	-	0.056	-
	249	-	-	0.056	-
Cfor3031	229	-	-	-	0.167
	231	-	-	-	0.033
	233	0.008	0.271	0.139	0.133
	235	0.078	0.129	0.083	0.167
	237	0.438	0.057	0.111	0.267
	239	0.380	0.514	0.556	0.167
	241	0.018	0.029	0.028	0.067
	243	-	-	0.056	-
	245	0.014	-	-	-
	247	0.064	-	0.028	-

Table 3. Genetic differentiation between populations as measured by F_{ST}. All comparisons have $P = 0.00$.

Population	Rangatira	Eglinton	Poor Knights
Mangere	0.13	0.25	0.14
Rangatira		0.15	0.09
Eglinton			0.06

Hardy-Weinberg equilibrium only in the Mangere Island population (Table 1), which could be explained by genetic admixture in this population.

Allele frequency distributions (Table 2) show that, with the exception of the Rangatira population, all populations possess private, or population-specific, alleles not found in other populations. Since the populations examined here represent different species and subspecies of *Cyanoramphus* parakeets, these private alleles could be lineage-specific. All Rangatira *Cfor* microsatellite alleles can be found in the Mangere Island population, but a number of alleles in the Mangere Island population (*Cfor0809*: 183bp allele; *Cfor1415*: 217bp and 225bp alleles; *Cfor2021*: 239bp allele; *Cfor2829*: 231bp allele; *Cfor3031*: 245bp and 247bp alleles) are not found on Rangatira. Apart from the 225bp *Cfor1415* allele and the 247bp *Cfor3031* allele, the rest of the above are also not found in any of the *C. auriceps* and *C. n. novaezelandiae* samples that we screened in this study. Although the differences in sample sizes can account for these observations, it is also possible that these alleles may be unique to the Forbes' parakeet population and perhaps may come exclusively from a Forbes' parakeet ancestry.

Pair wise comparison of F_{ST} values at the population level (Table 3) showed significant differentiation between all populations ($P = 0.00$ for all compari-

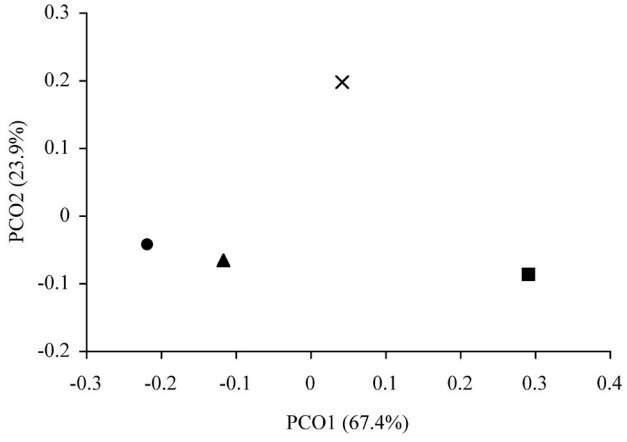

Figure 1. Principal coordinate analysis of genetic distance based on proportion of shared alleles (Bowcock et al. 1994) between Mangere Island (circle), Rangatira (triangle), Eglinton Valley (square), and Poor Knights Islands (cross) parakeet populations. Cumulatively, 91.3% of the total variation is represented by the two PCO axes.

sons). A higher level of genetic differentiation (F_{ST} = 0.25) is observed between Mangere Island parakeets and Eglinton Valley parakeets compared with that between Mangere Island parakeets and red-crowned parakeets on Rangatira (F_{ST} = 0.13) and Poor Knight Islands (F_{ST} = 0.14). Principal coordinate analysis of genetic distances (Figure 1) also indicates a closer relationship between Mangere Island parakeets and Rangatira parakeets as expected.

Discussion

Our preliminary screening of microsatellite DNA loci in four different parakeet populations showed that population or species specific alleles and differences in allele frequency distributions exist across the *Cyanoramphus* genus. More of these alleles are likely to be found when more populations and loci are tested. These features make the markers useful in population identification and assignment tests (for example, see MacAvoy *et al*. 2007 for similar tests in tuatara). Despite the small numbers of samples from Poor Knights Islands red-crowned parakeets and Eglinton Valley yellow-crowned parakeets that were made available for analysis, our data show private alleles and significant genetic differentiation (F_{ST} = 0.06, P = 0.00) can be detected between them and other populations studied. One might argue that this observation could merely reflect population level differences, rather than species level differences. However, phylogenetic evidence from allozymes (Triggs & Daugherty 1996) and mitochondrial DNA studies (Boon *et al*. 2000, 2001), together with ecological differences in habitat use and dietary preferences (Greene 1998) would lead to an expectation that there will be valid species level differences in microsatellite genotypes. Testing this hypothesis will require more populations and greater number of individuals to be screened. Nevertheless, we have taken the first step here to show that microsatellites are promising candidate markers for *Cyanoramphus* population genetic studies.

Screening of mitochondrial DNA haplotypes in the Mangere Island parakeet population (Ballantyne *et al*. 2004; Chan *et al*. 2006) suggested the majority of birds have the ancestral Forbes' parakeet haplogroup (haplogroup 3) using the classification of Boon *et al*. (2001), but microsatellite DNA analyses showed that a large number of these birds are in fact hybrids (Chan *et al*. 2006). In our genetic distance analysis presented here, it is apparent that the Mangere Island parakeet population is genetically closer to Rangatira Chatham Island red-crowned parakeet population than to the other two parakeet populations (Figure 1).

Since the isolation of microsatellite DNA markers from Forbes' parakeets was first reported (Chan *et al*. 2005), a number of new microsatellite loci have been isolated from other parrots species (for example, Adcock *et al*. 2005; Russello *et al*. 2007; Taylor & Parkin 2007). The potential of cross-species amplification of microsatellites (Primmer *et al*. 1996) suggests that some of these newer microsatellite markers could be adapted for use in *Cyanoramphus* to increase the resolution and robustness of further analyses.

In this study, we have shown that genetic analyses with microsatellite DNA markers provide informative data to our understanding of relationships between *Cyanoramphus* species, subspecies and populations. We believe that a comprehensive screening and analysis of *Cyanoramphus* populations using microsatellite DNA markers is likely to turn our fragmented knowledge of parakeet

evolution and population dynamics into a more comprehensive understanding of this genus.

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